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(54) Title: PLANT EXPANSIN PROMOTER SEQUENCES

(57) Abstract

Provided is a cotton *Gossypium hirsutum* promoter region from an expansin gene expressed in developing fiber.

PLANT EXPANSIN PROMOTER SEQUENCES

INTRODUCTION

Technical Field

This invention relates to a cotton expansin promoter encoding sequence, and its use in contructs useful in modifying cotton fiber phenotypes.

This invention particularly relates to methods of using *in vitro* constructed DNA transcription or expression cassettes capable of directing fiber-tissue transcription of a DNA sequence of interest in cotton to produce fiber cells having an altered phenotype, and to methods of providing for or modifying various characteristics of cotton fiber as well as the modified cotton fibers produced by the method.

Background

In general, genetic engineering techniques have been directed to modifying the phenotype of individual prokaryotic and eukaryotic cells, especially in culture. Plant cells have proven more intransigent than other eukaryotic cells, due not only to a lack of suitable vector systems but also as a result of the different goals involved. For many applications, it is desirable to be able to control gene expression at a particular stage in the growth of a plant or in a particular plant part. For this purpose, regulatory sequences are required which afford the desired initiation of transcription in the appropriate cell types and/or at the appropriate time

in the plant's development without having serious detrimental effects on plant development and productivity.

It is therefore of interest to be able to isolate sequences which can be used to provide the desired regulation
5   of transcription in a plant cell during the growing cycle of the host plant.

One aspect of this interest is the ability to change the phenotype of particular cell types, such as differentiated epidermal cells that originate in fiber tissue, *i.e.* cotton
10   fiber cells, so as to provide for altered or improved aspects of the mature cell type. Cotton is a plant of great commercial significance. In addition to the use of cotton fiber in the production of textiles, other uses of cotton include food preparation with cotton seed oil and animal feed
15   derived from cotton seed husks.

Despite the importance of cotton as a crop, the breeding and genetic engineering of cotton fiber phenotypes has taken place at a relatively slow rate because of the absence of reliable promoters for use in selectively effecting changes in
20   the phenotype of the fiber. In order to effect the desired phenotypic changes, transcription initiation regions capable of initiating transcription in fiber cells during development are desired. Thus, an important goal of cotton bioengineering research is the acquisition of a reliable promoter which would
25   permit expression of a protein selectively in cotton fiber to affect such qualities as fiber strength, length, color and dyability.

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Relevant Literature

Cotton fiber-specific promoters are discussed in PCT
publications WO 94/12014 and WO 95/08914, and John and Crow,
5   Proc. Natl. Acad. Sci. USA, 89:5769-5773, 1992. cDNA clones
that are preferentially expressed in cotton fiber have been
isolated. One of the clones isolated corresponds to mRNA and
protein that are highest during the late primary cell wall and
early secondary cell wall synthesis stages. John and Crow,
10   *supra.*

Agrobacterium-mediated cotton transformation is described
in Umbeck, United States Patents Nos. 5,004,863 and 5,159,135
and cotton transformation by particle bombardment is reported
in WO 92/15675, published September 17, 1992. Transformation
15   of *Brassica* has been described by Radke *et al.* (Theor. Appl.
Genet. (1988) 75;685-694; Plant Cell Reports (1992) 11:499-
505.

SUMMARY OF THE INVENTION

20   The invention provides a cotton (*Gossypium hirsutum*)
promoter region from an expansin gene expressed in developing
fiber. Novel DNA promoter sequences are supplied, and methods
for their use are described for directing transcription of a
gene of interest in cotton fiber using the promoter region
25   from an expansin gene which is expressed in cotton fibe.

In efforts to identify genes critical to fiber
development, we have initiated a program sequencing randomly
selected cDNA clones derived from a library prepared from mRNA

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harvested from fibers at the stage in which secondary wall

synthesis approaches its maximum rate (approximately 21 dpa).

We have characterized a cotton (*Gossypium hirsutum*) cDNA

clone which is a homolog of the expansin gene. The sequences

5   of this cDNA clone is homologous to that of other expansin

encoding sequences The 5' genomic promoter region from this

gene has been sequenced for approximately 2200 base pairs.

Thus, the application provides sequences and methods of

use relating to modification of phenotype in cotton fiber

10   using a promoter of the cotton expansin gene.

DESCRIPTION OF THE DRAWINGS

Figure 1. Nucleic acid sequences to approximately 2200

bases of the promoter region 5' to the encoding sequences to

15   the cotton fiber expansin gene.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the subject invention, novel

constructs and methods are described, which may be used

20   provide for transcription of a nucleotide sequence of interest

in cells of a plant host, preferentially in cotton fiber cells

to produce cotton fiber having an altered phenotype.

Cotton fiber is a differentiated single epidermal cell of

the outer integument of the ovule. It has four distinct

25   growth phases; initiation, elongation (primary cell wall

synthesis), secondary cell wall synthesis, and maturation.

Initiation of fiber development appears to be triggered by

hormones. The primary cell wall is laid down during the

elongation phase, lasting up to 25 days postanthesis (DPA).

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Synthesis of the secondary wall commences prior to the

cessation of the elongation phase and continues to

approximately 40 DPA, forming a wall of almost pure cellulose.

The constructs for use in such cells may include several

5   forms, depending upon the intended use of the construct.

Thus, the constructs include vectors, transcriptional

cassettes, expression cassettes and plasmids. The

transcriptional and translational initiation region (also

sometimes referred to as a "promoter,") contains the

10   transcriptional and translational functional elements and the

initiation control region derived from or obtainable from the

expansin gene (Figure 1).

In some embodiments, the promoter will be modified by the

addition of sequences, such as enhancers, or deletions of

15   nonessential and/or undesired sequences. By "obtainable" is

intended a promoter having a DNA sequence sufficiently similar

to that of a native promoter to provide for the desired

specificity of transcription of a DNA sequence of interest.

It includes natural and synthetic sequences as well as

20   sequences which may be a combination of synthetic and natural

sequences.

A transcriptional cassette for transcription of a

nucleotide sequence of interest in cotton fiber will include

in the direction of transcription, the cotton fiber

25   transcriptional initiation region from expansin, a DNA

sequence of interest, and a transcriptional termination region

functional in the plant cell. When the cassette provides for

the transcription and translation of a DNA sequence of

5

interest it is considered an expression cassette. One or more

introns may be also be present.

Other sequences may also be present, including those

encoding transit peptides and secretory leader sequences as

5 desired.

Downstream from, and under the regulatory control of, the

expansin transcriptional/translational initiation control

region is preferably a nucleotide sequence of interest which

provides for modification of the phenotype of fiber. The

10 nucleotide sequence may be any open reading frame encoding a

polypeptide of interest, for example, an enzyme, or a sequence

complementary to a genomic sequence, where the genomic

sequence may be an open reading frame, an intron, a noncoding

leader sequence, or any other sequence where the complementary

15 sequence inhibits transcription, messenger RNA processing, for

example, splicing, or translation. The nucleotide sequences

of this invention may be synthetic, naturally derived, or

combinations thereof. Depending upon the nature of the DNA

sequence of interest, it may be desirable to synthesize the

20 sequence with plant preferred codons. The plant preferred

codons may be determined from the codons of highest frequency

in the proteins expressed in the largest amount in the

particular plant species of interest. Phenotypic modification

can be achieved by modulating production either of an

25 endogenous transcription or translation product, for example

as to the amount, relative distribution, or the like, or an

exogenous transcription or translation product, for example to

provide for a novel function or products in a transgenic host

cell or tissue. Of particular interest are DNA sequences

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encoding expression products associated with the development

of plant fiber, including genes involved in metabolism of

cytokinins, auxins, ethylene, abscissic acid, and the like.

Methods and compositions for modulating cytokinin expression

5   are described in United States Patent No. 5,177,307, which

disclosure is hereby incorporated by reference.

Alternatively, various genes, from sources including other

eukaryotic or prokaryotic cells, including bacteria, such as

those from *Agrobacterium tumefaciens* T-DNA auxin and cytokinin

10   biosynthetic gene products, for example, and mammals, for

example interferons, may be used.

The termination region which is employed in the

expression cassette will be primarily one of convenience,

since the termination regions appear to be relatively

15   interchangeable. The termination region may be native with

the transcriptional initiation region, may be native with the

DNA sequence of interest, may be derived from another source.

The termination region may be naturally occurring, or wholly

or partially synthetic. Convenient termination regions are

20   available from the Ti-plasmid of *A. tumefaciens,* such as the

octopine synthase and nopaline synthase termination regions.

In some embodiments, it may be desired to use the 3'

termination region native to the cotton fiber transcription

initiation region used in a particular construct.

25   As described herein, in some instances additional

nucleotide sequences will be present in the constructs to

provide for targeting of a particular gene product to specific

cellular locations.

7

A variety of techniques are available and known to those skilled in the art for introduction of constructs into a plant cell host. These techniques include transfection with DNA employing *A. tumefaciens* or *A. rhizogenes* as the

5   transfecting agent, protoplast fusion, injection, electroporation, particle acceleration, etc. For transformation with *Agrobacterium*, plasmids can be prepared in *E. coli* which contain DNA homologous with the Ti-plasmid, particularly T-DNA. The plasmid may or may not be capable of

10   replication in *Agrobacterium*, that is, it may or may not have a broad spectrum prokaryotic replication system such as does, for example, pRK290, depending in part upon whether the transcription cassette is to be integrated into the Ti-plasmid or to be retained on an independent plasmid. The

15   *Agrobacterium* host will contain a plasmid having the *vir* genes necessary for transfer of the T-DNA to the plant cell and may or may not have the complete T-DNA. At least the right border and frequently both the right and left borders of the T-DNA of the Ti- or Ri-plasmids will be joined as flanking regions to

20   the transcription construct. The use of T-DNA for transformation of plant cells has received extensive study and is amply described in EPA Serial No. 120,516, Hoekema, In: The Binary Plant Vector System Offset-drukkerij Kanters B.V., Alblasserdam, 1985, Chapter V, Knauf, *et al.*, Genetic Analysis

25   of Host Range Expression by *Agrobacterium*, In: Molecular Genetics of the Bacteria-Plant Interaction, Puhler, A. ed., Springer-Verlag, NY, 1983, p. 245, and An, *et al.*, *EMBO J.* (1985) 4:277-284.

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For infection, particle acceleration and electroporation, a disarmed Ti-plasmid lacking particularly the tumor genes found in the T-DNA region) may be introduced into the plant cell. By means of a helper plasmid, the construct may be

5   transferred to the *A. tumefaciens* and the resulting transfected organism used for transfecting a plant cell; explants may be cultivated with transformed *A. tumefaciens* or *A. rhizogenes* to allow for transfer of the transcription cassette to the plant cells. Alternatively, to enhance

10   integration into the plant genome, terminal repeats of transposons may be used as borders in conjunction with a transposase. In this situation, expression of the transposase should be inducible, so that once the transcription construct is integrated into the genome, it should be relatively stably

15   integrated. Transgenic plant cells are then placed in an appropriate selective medium for selection of transgenic cells which are then grown to callus, shoots grown and plantlets generated from the shoot by growing in rooting medium.

To confirm the presence of the transgenes in transgenic

20   cells and plants, a Southern blot analysis can be performed using methods known to those skilled in the art. Expression products of the transgenes can be detected in any of a variety of ways, depending upon the nature of the product, and include immune assay, enzyme assay or visual inspection, for example

25   to detect pigment formation in the appropriate plant part or cells. Once transgenic plants have been obtained, they may be grown to produce fiber having the desired phenotype. The fibers may be harvested, and/or the seed collected. The seed may serve as a source for growing additional plants having the

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desired characteristics. The terms transgenic plants and transgenic cells include plants and cells derived from either transgenic plants or transgenic cells.

The various sequences provided herein may be used as
5   molecular probes for the isolation of other sequences which may be useful in the present invention, for example, to obtain related transcriptional initiation regions from the same or different plant sources. Related transcriptional initiation regions obtainable from the sequences provided in this
10   invention will show at least about 60% homology, and more preferred regions will demonstrate an even greater percentage of homology with the probes.

Of particular importance is the ability to obtain related transcription initiation control regions having the timing and
15   tissue parameters described herein. Thus, by employing the techniques described in this application, and other techniques known in the art (such as Maniatis, *et al.*, *Molecular Cloning, - A Laboratory Manual* (Cold Spring Harbor, New York) 1982), other encoding regions or transcription initiation
20   regions of expansin as described in this invention may be determined. The constructs can also be used in conjunction with plant regeneration systems to obtain plant cells and plants; thus, the constructs may be used to modify the phenotype of fiber cells, to provide cotton fibers which are
25   colored as the result of genetic engineering to heretofor unavailable hues and/or intensities.

Various varieties and lines of cotton may find use in the described methods. Cultivated cotton species include *Gossypium hirsutum* and *G. babadense* (extra-long stable, or

10

Pima cotton), which evolved in the New World, and the Old

World crops G. *herbaceum* and *G. arboreum*.

5   EXAMPLES

The following examples are offered by way of illustration

and not by limitation.

                                    Example 1

                                   cDNA libraries

10   An unamplified cDNA library was used to prepare the

Lambda Uni-Zap vector (Stratagene, LaJolla, CA) using cDNA

derived from polyA+ mRNA prepared from fibers of *Gossypium*

hirsutum Acala SJ-2 harvested at 21 DPA, the time at which

secondary wall expansin is approaching a maximal rate (13).

15   Approximately 250 plaques were randomly selected from the cDNA

library, phages purified and plasmids excised from the phage

vector and transformed.

The resulting clones/inserts were size screened on 0.8%

agarose gels (DNA inserts below 600bp were excluded).

20

                                    Example 2

                   Isolation and Sequencing of cDNA Clones

Plasmid DNA inserts were randomly sequenced using an

Applied Biosystems (Foster City, CA) Model 373A DNA sequencer.

25

                                    Example 3

                      Northern and Southern Analyses.

Cotton plants (*G. hirsutum* cv. Coker 130) were grown in

the greenhouse and tissues harvested at the appropriate times

                                        11

indicated and frozen in liquid N$_2$. Total cotton RNA and

cotton genomic DNA was prepared and subjected to Northern and

Southern analyses as described previously (14).

5   Example 4

              Identification, Differential Expression and

              Genomic Analysis of Cotton Expansin Genes

         During the course of screening and sequencing random cDNA

    clones from a cotton fiber specific cDNA library, it was

10 discovered that one cDNA clone was very active during primary

    cell wall developement and had homology to the protein encoded

    by the expansin genes.

         This clone was then utilized as a probe for Northern blot

    analysis to determine the differential expression in cotton

15 tissues and developing cotton fiber. The expansin gene

    encodes a mRNA which is expressed at high levels in developing

    fiber, beginning at approximately day 1 through primary cell

    wall development at approximately day 20 post anthesis.

20   Example 5

                         Genomic DNA

         cDNA for the expansin clone was used to probe for genomic

    clones. Full length genomic DNA was obtained from a library

    made using the lambda dash 2 vector from Stratagene™, which

25 was used to construct a genomic DNA library from cotton

    variety Coker 130 (*Gossypium hirsutum* cv. coker 130), using

    DNA obtained from germinating seedlings.

         The cotton genomic library was probed with a expansin

    probe and genomic phage candidates were identified and

                              12

purified. Figure 1 provides an approximately 2200 base pair

sequence of the expansin promoter region which is immediately

5' to the expansin encoding region. The start of the expansin

enzyme encoding region is at the ATG at base number 2297, and

5   the genomic clone begins at base number 122 of Figure 1.

Example 6

Cotton Transformation

10   Construct Preparation

Promoter constructs comprising the expansin promoter

sequences linked to a gene of interest and other genetic

elements of interest can be prepared in any of a number of

ways known to the art, such as by ligation.

15

Explant Preparation

Coker 315 seeds are surface disinfected by placing in 50%

Clorox (2.5% sodium hypochlorite solution) for 20 minutes and

rinsing 3 times in sterile distilled water. Following surface

20   sterilization, seeds are germinated in 25 x 150 sterile tubes

containing 25 mls 1/2 x MS salts: 1/2 x B5 vitamins: 1.5%

glucose: 0.3% gelrite. Seedlings are germinated in the dark

at 28°C for 7 days. On the seventh day seedlings are placed in

the light at 28±2°C.

25

Cocultivation and Plant Regeneration

Single colonies of A. tumefaciens strain 2760 containing

binary plasmids pCGN2917 and pCGN2926 are transferred to 5 ml

of MG/L broth and grown overnight at 30°C. Bacteria cultures

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are diluted to 1 x 10^8 cells/ml with MG/L just prior to
cocultivation. Hypocotyls are excised from eight day old
seedlings, cut into 0.5-0.7 cm sections and placed onto
tobacco feeder plates (Horsch *et al.* 1985). Feeder plates are
5   prepared one day before use by plating 1.0 ml tobacco
suspension culture onto a petri plate containing Callus
Initiation Medium CIM without antibiotics (MS salts: B5
vitamins: 3 % glucose: 0.1 mg/L 2,4-D: 0.1 mg/L kinetin: 0.3%
gelrite, pH adjusted to 5.8 prior to autoclaving). A sterile
10   filter paper disc (Whatman #1) was placed on top of the feeder
cells prior to use. After all sections are prepared, each
section was dipped into an *A. tumefaciens* culture, blotted on
sterile paper towels and returned to the tobacco feeder
plates.

15   Following two days of cocultivation on the feeder plates,
hypocotyl sections are placed on fresh Callus Initiation
Medium containing 75 mg/L kanamycin and 500 mg/L
carbenicillin. Tissue is incubated at 28±2°C, 30uE 16:8
light:dark period for 4 weeks. At four weeks the entire
20   explant is transferred to fresh callus initiation medium
containing antibiotics. After two weeks on the second pass,
the callus is removed from the explants and split between
Callus Initiation Medium and Regeneration Medium (MS salts:
40mM KNO$_3$: 10 mM NH$_4$Cl:B5 vitamins:3% glucose:0.3% gelrite:400
25   mg/L carb:75 mg/L kanamycin).

        Embryogenic callus is identified 2-6 months following
initiation and was subcultured onto fresh regeneration medium.
Embryos are selected for germination, placed in static liquid
Embryo Pulsing Medium (Stewart and Hsu medium: 0.01 mg/l NAA:

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0.01 mg/L kinetin: 0.2 mg/L GA3) and incubated overnight at 30°C. The embryos are blotted on paper towels and placed into Magenta boxes containing 40 mls of Stewart and Hsu medium solidified with Gelrite. Germinating embryos are maintained at

5   28±2°C 50 uE $m^{-2}s^{-1}$ 16:8 photoperiod. Rooted plantlets are transferred to soil and established in the greenhouse.

Cotton growth conditions in growth chambers are as follows: 16 hour photoperiod, temperature of approximately 80-85°, light intensity of approximately 500µEinsteins. Cotton

10   growth conditions in greenhouses are as follows: 14-16 hour photoperiod with light intensity of at least 400µEinsteins, day temperature 90-95°F, night temperature 70-75°F, relative humidity to approximately 80%.

15   Plant Analysis

Flowers from greenhouse grown T1 plants are tagged at anthesis in the greenhouse. Squares (cotton flower buds), flowers, bolls etc. are harvested from these plants at various stages of development and assayed for observable phenotype or

20   tested for enzyme activity.

The above results demonstrate how the expansin cDNA may be used to alter the phenotype of a transgenic plant cell, and how the promoter may be used to modify transgenic cotton fiber

25   cells.

All publications and patent applications cited in this specification are herein incorporated by reference as if each

15

individual publication or patent application are specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail, by way of illustration and example for purposes of clarity and understanding, it will be readily apparent to those of ordinary skill in the art that certain changes and modifications may be made thereto, without departing from the spirit or scope of the appended claims.

CLAIMS

What is claimed is:

1. An isolated DNA sequence to a plant expansin

5   promoter region of cotton.

2. The promoter encoding sequence of Claim 1

wherein said cotton expansin promoter region is taken the

from sequence of Figure 1.

3. A recombinant DNA construct comprising any of

10   the DNA encoding sequences of Claims 1-2.

4. A plant cell comprising a DNA construct of Claim 3.

5. A plant comprising a cell of Claim 4.

6. A method of modifying fiber phenotype in a

cotton plant, said method comprising:

15   transforming a plant cell with DNA comprising a

construct having the promoter of Claim 3 linked to a gene

of interest,

wherein said gene of interest is capable of

modifying a fiber characteristic when expressed in a

20   fiber cell.

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FIG. 1A

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          740        760        780        800
           *          *          *          *
ACTACAACCCTAAATGCTTTATAGGCGCATAGATCATGGGTTTAGCTTTGGATCCCCATAAAGTACAAATACTGAGGTTCTT

          820        840        860        880
           *          *          *          *
TAGTGAATGATCATGCATGGACACATGATGTCTCTTTTTAGGCATTGACAAACTCGTCATTTTTTCATACAAATTTCTTT

          900        920        940        960
           *          *          *          *
GGTCAATTAAATTTTCCATTAAATTAGGAAACCGCCCTCCAAAATTATANTGGTAACGGTGGAAGGNTTATCCAANTTCG

          980       1000       1020       1040
           *          *          *          *
ACCATTCGAACCACTTTTAAAAAATTAGAAAGTTATAATTTTTTTTTTCAAAAAACTATAATACCTCTCTAGTTTTAGCT

         1060       1080       1100       1120
           *          *          *          *
AATTAAATTATTATTATTTTATTGTTATTAAAAGTGTAACTTGCACTCAACTATTAGTAAGTTTACGTTTTG

         1140       1160       1180       1200
           *          *          *          *
ATCACTTAATTTCAGAAAGTTAAAAAATGGTCTTTGAACTATTCGAAAATTTCATTTAAGTTACTGGAATATTTAAAAG

         1220       1240       1260       1280
           *          *          *          *
TTTTTATTTAAGTCACCGGGCTATTAAGTTTTTTTTTTTAAAAATTCGATTAGCAAGTTCCAAGCTACGATTCGATAAGTGA

         1300       1320       1340       1360
           *          *          *          *
TACAAATGGATTATACTTATTGACAAATAGAATATACATTAGGTCCAAGTTGATATTACGGTCAGTGTTGAAAATCGAAA

         1380       1400       1420       1440
           *          *          *          *
AAAAAATATTTGGATTTGATTCATAAATTTATGACTTCAAAGCTGGTTCATGAAAAAGAACTAAAGTGTAGGAGGGAAGG
```

FIG. 1B

```
      1460      1480      1500      1520
         *         *         *         *
AAAAAAATATCTTTTGATTGGCACAAACAGTGCGAACAAAGAGACCACACAATAACAATTTTAACAATATACTAATTTA

      1540      1560      1580      1600
         *         *         *         *
AATGAAAAATTTTCAATAATTTAATAAGTTAACCGAGGAAAACTTACTAAGAGTTAGTTACCCCTGTTAAAATAACTTTC

      1620      1640      1660      1680
         *         *         *         *
ATGAAGTAATAGAAACTTTTAGTACGTATCATCTTATATAGAACAATTTCTATTTCAGAAAGTCAAGAAAATTGTATTC

      1700      1720      1740      1760
         *         *         *         *
TAGAAAATGGCGACTTCTTCACCTTCAGTCCTTCCCTGATCGGCGCTTGTGAAAAACGAAAAACCTGAGTCTGATTGGCT

      1780      1800      1820      1840
         *         *         *         *
GACTGAAAATGAACCTACTCATCACCATTCACTATTACCAACTTCAAATGATAGGGGAATTAACTGGTAAAGTGTAACTC

      1860      1880      1900      1920
         *         *         *         *
CACCGATGGTTGAGGTGGTTGGCTGGAGTTAAATGAGATTTTTTGTTTCAAGTGGCTTCAATTGCAAGCAATT

      1940      1960      1980      2000
         *         *         *         *
AGGAGACTGCGCTGGAATAACCCCTCGCTCAACCTTCCGCCATTGTTATGGTTTAATTAAACATTATGTTTCCATCCATC

      2020      2040      2060      2080
         *         *         *         *
TATATTTATATCCATTAAAACAAGTCGTTGAGCAAATAATGGATACTGGATACCATCATATCTATGATTAAAATTTTGCA
```

FIG. 1C

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         2100        2120        2140        2160
           *    *      *    *      *    *      *    *
TGTGCCCTTTTAAATGTATAGCTTAAGCCTTAATTATCCTCCAAATTTGTACTCTTTCACCACTTAATTAGCTACGTACGG

         2180        2200        2220        2240
           *    *      *    *      *    *      *    *
TACTTAGCGGTTGCTTGTCATCTTCTGTACTACACAAACTCTTTCTCATTTGTATAAATAGCTATACACTTTTTCTCTCCTC

         2260        2280        2300        2320
           *    *      *    *      *    *      *    *
AAATCAATAAGGTTAGGTCAGCCAATTGTTTGAGCTAGCTCTTACTCAAATGGCAACCAAAACGATGATGTTGCAA

         2340        2360        2380        2400
           *    *      *    *      *    *      *    *
ATATTTCCACTTTCTTCTTTTTGTTCAGTGTCTGCAACTCCATTTCCTTGGTGCTAATGGAGATGACAATGGTGGTTG

         2420        2440        2460        2480
           *    *      *    *      *    *      *    *
GCAAACTGCCCATGCCACCTTCTACGGTGGTGCTGATGCTACCGGCACAATGGGTGAGTTTCAAACTTTCAAAACCATTAC

         2500        2520        2540        2560
           *    *      *    *      *    *      *    *
GGCATTACCTACATAAAAATCTCTAGGCTATGTTCTTAATTGTGATGTTCTCTATAGGGGGAGCTTGTGGTTATGGAAA

         2580        2600
           *    *      *    *
CCTGTACAGTCAAAGCCGAATTCCAGCACACTGGCGCGCCGTTACTAGTGGA
```

FIG. 1D

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   C12N15/29   C12N15/82   C12N5/10   A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   C12N   A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 495 070 A (JOHN MALIYAKAL) 27 February 1996 see sequence ID no. 26 see column 63 – column 66 ---	1-6
A	SHIMIZU, YOSHINORI ET AL: "Changes in levels of mRNAs for cell wall-related enzymes in growing cotton fiber cells" PLANT CELL PHYSIOL., vol. 38, no. 3, 1997, pages 375-378, XP002064957 see page 377, column 2 see figure 3B --- -/--	

| X | Further documents are listed in the continuation of box C. | | X | Patent family members are listed in annex. |

Date of the actual completion of the international search	Date of mailing of the international search report
14 May 1998	29/05/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Chakravarty, A

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document. with indication.where appropriate. of the relevant passages	Relevant to claim No.
A	JOHN, MALIYAKAL E: "Gene expression in cotton (Gossypium hirsutum) fibers" ADV. PLANT BIOTECHNOL. BIOCHEM., `PROC. INT. CONF. BIOTECHNOL. AGRIC. FOR.! (1993), 27-32. EDITOR(S): LODAH, M. L. PUBLISHER: IND. SOC. AGRIC. BIOCHEM., KANPUR, INDIA. CODEN: 60RTAG, XP000610459 see the whole document	1-6
A	WO 95 08914 A (AGRACETUS) 6 April 1995 see page 8, line 13 - page 23, line 25	1-6
A	WO 96 40924 A (CALGENE INC ;MCBRIDE KEVIN (US); STALKER DAVID M (US); PEAR JULIE) 19 December 1996 see the whole document	1-6
A	WO 96 40951 A (CALGENE INC) 19 December 1996 see the whole document	1-6
A	WO 96 35442 A (PENN STATE RES FOUND) 14 November 1996 see the whole document	1-6

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Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 5495070	A	27-02-1996	US 5521078 A		28-05-1996
			US 5620882 A		15-04-1997
			US 5597718 A		28-01-1997
WO 9508914	A	06-04-1995	AU 7925094 A		18-04-1995
			EP 0670670 A		13-09-1995
			JP 8509871 T		22-10-1996
			US 5608148 A		04-03-1997
WO 9640924	A	19-12-1996	AU 6269196 A		30-12-1996
			EP 0835311 A		15-04-1998
WO 9640951	A	19-12-1996	AU 6330496 A		30-12-1996
			EP 0833932 A		08-04-1998
WO 9635442	A	14-11-1996	AU 4026295 A		04-04-1996